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13. SUPPLEMENTARY NOTES

14. ABSTRACT
We have acquired the femtosecond laser system Tsunami from Spectra Physics, CA, ($89,900) pumped by a DPSS laser Millennia($54,500) , also from Spectra Physics, CA, and used them for developing multi-photon process based technologies. It is known that two-photon excitation produces highly localized effect near the focal point when the pump beam is focused by a lens. This property can be used to achieve excellent depth resolution to create a highly localized two-photon initiated process at different depths, with a very well defined depth control. This feature provides an unique opportunity to develop such diverse applications as for 3D volume imaging, 3D optical circuitry fabrication, high density 3D optical data storage, etc. In this context we were using the newly acquired laser system (Ultra-short pulse, high repetition rate Ti:Sapphire laser, Tsunami pumped by a diode pumped solid state laser, Millenia) for two-photon confocal microscopy and spectroscopy, two-photon initiated micro-fabrication and two-photon optical datastorage,. We were able to use this new laser system with our confocal micro-spectroflourimeter as well as for fabricating 3D optical circuitry.

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Instruments acquired and Justification:

We have acquired a tunable femto-second Ti:Sapphire laser, Tsunami from Spectra-Physics,CA(\$89,900) pumped by a diode pumped solid state Laser Millennia from Spectra-Physics, CA (\$54,500) . The acquired laser can produce high repetition rate femto-second pulses which can be used for the study of multi-photon processes. This laser with its tunability and ultra short pulses, was ideal for our work on multi-photon microscopy, mutli-photon process based 3D microfabrication of optical channel circuitry as well as the two-photon process based 3D optical data-storage.

Summary of Research Projects for which the equipment was used:

Two-photon excitation produces highly localized effect near the focal point when the pump beam is focussed by a lens. This property can be used to achieve excellent depth resolution to create a highly localized two-photon initiated process at different depths, with a very well defined depth control. We therefore have well defined volume access by optical sectioning of a medium. This feature provides an unique opportunity to develop such diverse applications as for 3D volume imaging, 3D optical circuitry fabrication, high density 3D optical data storage, etc. In this context we were using the newly acquired laser system (Ultra-short pulse, high repetition rate Ti:Sapphire laser, Tsunami pumped by a diode pumped solid state laser, Millenia) for two-photon confocal microscopy and spectroscopy, two-photon initiated micro-fabrication and two-photon optical datastorage,. Some examples are given here.

3D micro-fabrication:

A novel approach was used for micro-fabrication of three-dimensional optical circuitry using in-situ two-photon assisted polymerization in an as-formed bulk sample. The bulk media consisted of a blend of photo-curable and thermally curable epoxies. 1x2 and 1X4 splitters were fabricated inside the volume and imaged by confocal microscopy. End-fire coupling of a He-Ne laser beam into these splitters was also achieved. By controlling the size and shape of the waveguides, we were able to make single mode or multimode waveguides.

For our studies we used a mixture of UV curable epoxy NOA 72(from Norland Optical Norland Products Inc., New Jersey, USA.) and Epo-tek301(from Epoxy

Technology, Inc. Massachusetts, USA) and a two-photon chromophore AF183 (6-benzothiazol-2-yl(2-naphtyl))diphenylamine synthesized at Airforce Research Laboratory). The mixture of UV curable epoxy, thermal epoxy and the chromophore was spread over a glass slide and after curing for 24 hours at room temperature formed a thick film of thickness around 100-150 μm.

For the fabricating of waveguide channels in the bulk, we used 800 nm, ~100 fs pulses from a Ti:Sapphire laser(Tsunami from Spectra Physics). The average power of the laser was ~ 1W. After attenuators and beam expansion, the average power incident over the sample was reduced to 40mW. The details of the setup used for the fabrication was similar to that was reported earlier. We have used 60X oil immersion objective (NA 1.4, from Nikon) for focussing the laser beam on to the sample, after expanding the laser beam to utilize the full NA of the objective. The written channels were found to be asymmetric along YZ plane(elongated along Z direction), even though the usage of high NA objective reduced the asymmetry. To achieve a symmetrical channel we have written the channels 3-5 times by slightly translating the sample in X or Y direction. This way we were able to fabricate comparatively symmetric channels.

The fabricated channel waveguide's geometry and location were monitored using fluorescence confocal microscopy. After the two-photon induced photoprocess, the dye AF183 show a shift in the excitation and fluorescence properties and this can be utilized to image the written channels using confocal laser scanning microscope(MRC1024 from Bio-Rad). The confocal microscopy images presented in this paper are acquired using an excitation of 488nm line from a Kr:Argon laser.

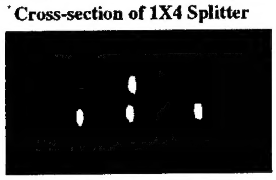
The advantage of this rapid laser prototyping technique is the easiness of crating 3-dimensional waveguides and other optical circuit creation. To demonstrate this we have written series of vertically stacked channels . The confocal images as well as the nearfiled images of the coupled laserlight from the channel waveguides are shown in the figure below (fig.1).

Fig 1

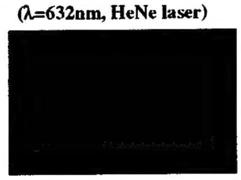
- Confocal Microscope Image of 1X4 Splitter

single channel region Branching region 4-splitted branches

- Confocal Microscope Image of Cross-section of 1X4 Splitter



- Output of Guiding Mode (λ=632nm, HeNe laser)



In conclusion, we have used two-photon absorption based laser rapid prototyping to fabricate single mode and multimode channel waveguides as well as 1x4 beam splitters inside a pre-gelled bulk. We have also verified the mode structure of these channel waveguides by coupling laser into these channels and observing the output modes.

Confocal Localized spectrometer:

We have developed our own system of Two-photon Laser Scanning Micro-spectrofluorometer (TPLSMF) for the purpose of multi-color spectral imaging. With one-photon or two-photon confocal laser scanning microscopy, images are acquired through broad band emission filters, which cannot distinguish between small changes in

Fig. 2

- Stacked Channel Waveguide

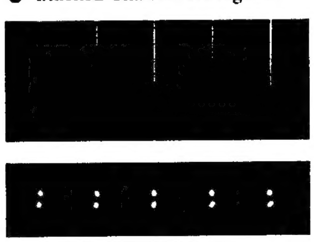


Figure. Confocal microscope images of single-mode channel waveguides which were stacked vertically with a separation of 30 μm.

Optical Loss = 0.8 dB/cm
for a single mode channel waveguide (by cut-back method)

- Output Mode Pattern

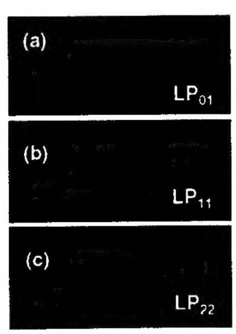


Figure. Near field intensity distributions of guided modes at λ=632nm. (a) single mode and (b), (c) multi mode channel waveguides.

the spectral profiles of the sample. To get a complete picture of the sample, their emission spectra also have to be monitored. In order to acquire the spectral images, we have developed a confocal/Multiphoton localized spectrometer, which has the spatial resolution comparable to the confocal/Multiphoton microscope as well as a spectral resolution of ~1nm. TPLSM, TPLSMF has inherent localization ability and much less photodamage compared with one-photon system. Because excitation is limited to the focus point, TPLSMF can provide spectral resolution of the diffraction-limited spot size without any pinhole. This feature makes it very promising in studying the dynamic changes inside the living cells. In contrast, in one-photon confocal micro-spectrofluorometry, researchers have to reduce the aperture to get localized spectra, which is in fact a tradeoff between signal levels and spatial resolution. Therefore, it is hard to reach the theoretical spatial resolution of using one-photon confocal micro-spectrofluorometer.

Spectrograph consists of a fiber (1 mm, multi-mode) coupled monochromator (Holoscope from Keiser Inc.), equipped with a cooled CCD (Princeton Instruments) as detector. The Setup for Two-photon Laser Scanning Microspectrofluorometry (TPLSMF) is shown as Figure 3.

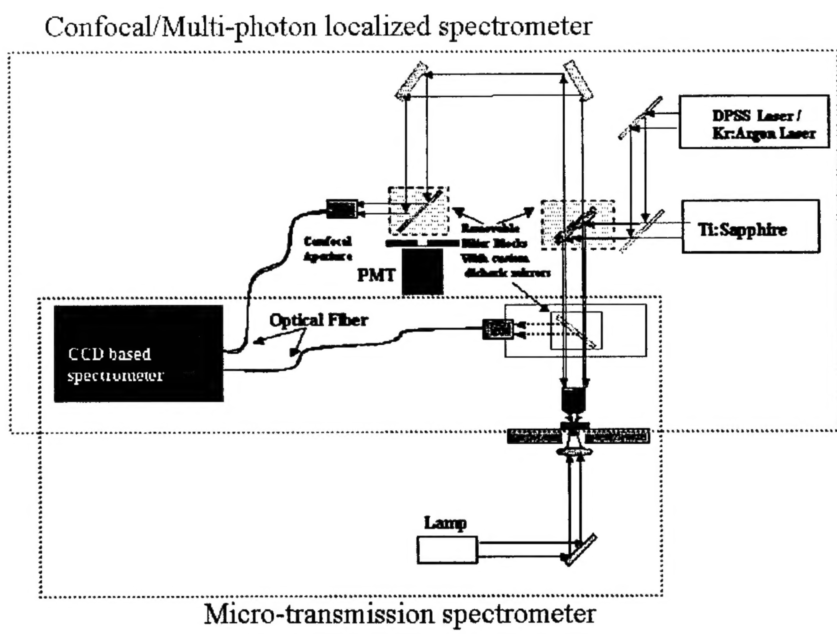


Fig. 3 Schematic diagram of the confocal/Multiphoton localized spectrometer and micro-transmission spectrometer. This fiber coupled localized spectrometer was built upon the existing confocal microscope

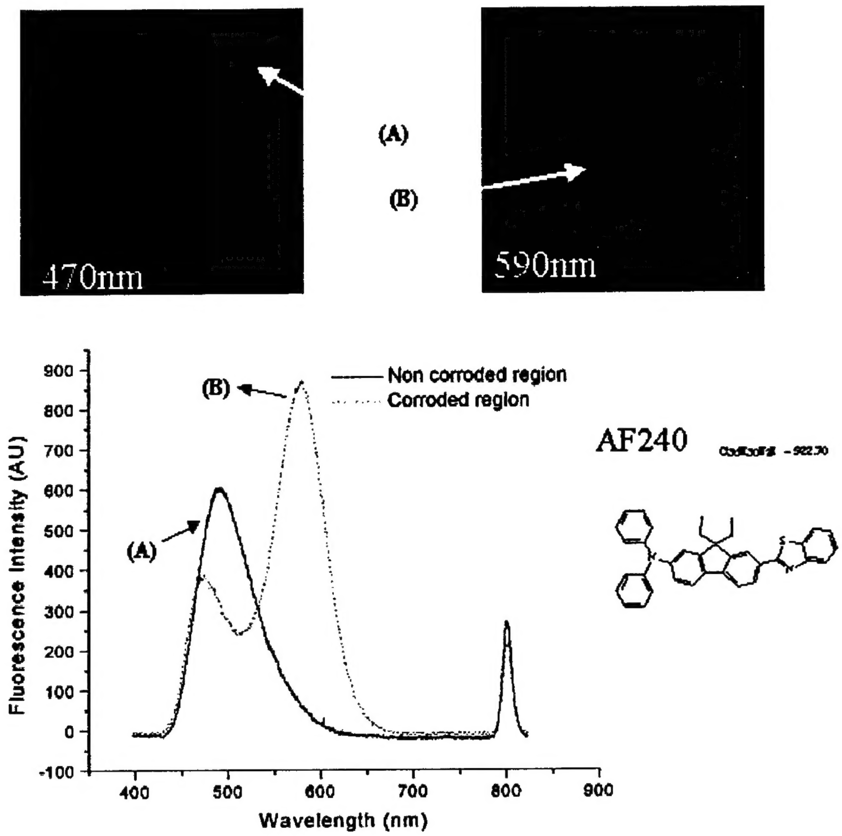


Fig. 4. Polymeric paint coatings doped with tow-photon chromophore AF-240, was made on aluminum substrates jept in acidic environment. Two-Photon confocal spectrally resolved images and localized spectra taken at two different points are shown here. Spectra are taken from aregion of the size ~ 10 μm X 10μm.

The fluorescence acquired under the microscope was directed to a fiber by a dichoric mirror without passing any filters inside the scanhead. An absorption filter in the IR range was used inside the spectrograph to cut off the excitation lines from laser.

Fig. 4 shows an image and spectra taken using this versatile confocal microscope and localized spectrometer o a polymeric paint film (at a depth of ~50 μm from surface, i.e, ~2 times the MFP of the paint film.). The change in fluorescence as well as the spectrally resolved imaging clearly shows the penetration of acidic environment into the film. This is a potential tool to detect the onset of corrosion due to acidic or other environmental changes.

Our study demonstrates that confocal microscopy can be used as a tool for nondestructive evaluation of multilayer coatings and inspections of any corrosion on the underlying surface without removing or peeling off the paint layer. We were able to non-destructively study the onset of damages in paint coatings using confocal microscopy(two-photon fluorescence and reflection mode).

So with a combination of confocal/Multiphoton gated imaging, and the study of the change in spectral profile using a confocal/Multiphoton localized spectrometer, one can study the effect of change in environmental conditions on a pigmented polymeric paint coating, or the onset of corrosion of a metallic substrate through the coating.